Sequential feeding using whole wheat and a separate protein-mineral concentrate improved feed efficiency in laying hens

M. Umar Faruk,*† I. Bouvarel,‡ N. Même,* N. Rideau,* L. Roffidal,§ H. M. Tukur,† D. Bastianelli,# Y. Nys,* and P. Lescoat*[1]

*INRA, UR83 Recherches Avicoles, F-37380 Nouzilly, France; †Department of Animal Science, Usman Danfodio University, PMB 2346, Sokoto, Nigeria; ‡Institut Technique de l'Aviculture (ITAVI), F-37380 Nouzilly, France; §INZO, 1 rue Marebaudière, F-35760 Montgermont, France; and #Service d'alimentation animale, Cirad, Systèmes d'élevage, Baillarguet TA C-18/A, F-34398 Montpellier Cedex 05, France

ABSTRACT The effect of feeding nutritionally different diets in sequential or loose-mix systems on the performance of laying hen was investigated from 16 to 46 wk of age. Equal proportions of whole wheat grain and protein-mineral concentrate (balancer diet) were fed either alternatively (sequential) or together (loose-mix) to ISA Brown hens. The control was fed a complete layer diet conventionally. Each treatment was allocated 16 cages and each cage contained 5 birds. Light was provided 16 h daily (0400 to 2000 h). Feed offered was controlled (121 g/bird per d) and distributed twice (4 and 11 h after lights-on). In the sequential treatment, only wheat was fed at first distribution, followed by balancer diet at the second distribution. In loose-mix, the 2 rations were mixed and fed together during the 2 distributions. Leftover feed was always removed before the next distribution. Sequential feeding reduced total feed intake when compared with loose-mix and control. It had lower wheat (−9 g/bird per d) but higher balancer (+1.7 g/bird per d) intakes than loose-mix. Egg production, egg mass, and egg weight were similar among treatments. This led to an improvement in efficiency of feed utilization in sequential compared with loose-mix and control (10 and 5%, respectively). Birds fed sequentially had lower calculated ME (kcal/bird per d) intake than those fed in loose-mix and control. Calculated CP (g/bird per d) intake was reduced in sequential compared with loose-mix and control. Sequentially fed hens were lighter in BW. However, they had heavier gizzard, pancreas, and liver. Similar liver lipid was observed among treatments. Liver glycogen was higher in loose-mix than the 2 other treatments. It was concluded that feeding whole wheat and balancer diet, sequentially or loosely mixed, had no negative effect on performance in laying hens. Thus, the 2 systems are alternative to conventional feeding. The increased efficiency of feed utilization in sequential feeding is an added advantage compared with loose-mix and thus could be employed in situations where it is practicable.

Key words: laying hen, sequential feeding, loose-mix feeding, whole wheat, feeding system

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INTRODUCTION

Laying hens are commonly fed a single complete diet. This system has the advantage of uniformity of the diet. One disadvantage is the need for grinding the main dietary components. Energy required for grinding comprises between 25 and 30% of feed manufacturing (Dozier, 2002). It was known that the poultry digestive system is capable of digesting whole grain. Therefore, it is logical to think that the cost incurred in grinding and handling of cereals will be significantly reduced if birds are fed whole grains. Furthermore, the amount of gas emissions due to grinding and transportation could be reduced. In countries in which the cost of transport and diet mixing is expensive, it may be more economical to transport only a protein concentrate. In addition, it allows the use of locally grown cereals in the farm.

Whole grains can be fed with a protein concentrate in different systems (Noirot et al., 1998): simultaneously in different containers (choice feeding), mixed together and fed in single container (loose-mix), or fed at different times of the day (sequential). Choice feeding using unground cereals is accompanied by an improvement in feed utilization because it allows a degree of feed selection by the animal. It presents, however, the inconvenience of having more than one feeding trough to contain the different diets. As such, it is less prac-

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[1]Corresponding author: lescoat@tours.inra.fr

tical in application. Loose-mix and sequential feeding could be practical because only 1 diet and container are required at a time. However, a feeding system using whole grains without reducing bird performance is yet to be developed.

Sequential feeding had been reported to increase total intake when birds were fed a mixture of whole cereals and protein concentrate sequentially (Blair et al., 1973). Egg production and weight were, however, not affected, thereby decreasing efficiency of feed utilization of these birds. Conversely, low feed intakes (Leeson and Summers, 1978; Reichmann and Connor, 1979; Robinson, 1985; Lee and Ohh, 2002) were observed when hens were fed sequentially. Egg production was similar (Leeson and Summers, 1978; Reichmann and Connor, 1979; Lee and Ohh, 2002) or reduced (Robinson, 1985) compared with the conventional feeding system. All of the above authors observed low egg weight in birds fed sequentially. The limited information on loose-mix (Lee and Ohh, 2002) indicated that it reduced feed intake but resulted in similar egg production with a slight decrease in egg weight compared with the conventional system.

Combination of the above studies indicates a broad variation of performance in terms of feed intake, egg production, egg weight, and efficiency of feed conversion in sequentially fed birds compared with conventional ones. With genetic improvement, it could be asked if today's birds are better able to adjust their intake and performance when fed different diets sequentially. The above studies fed diets that contrasted greatly in energy, protein, and calcium. In addition, most experiments gave ad libitum access to these diets and allowed birds to regulate their intake. However, hens might not adapt their intake to fit with their requirements, thereby resulting in some inconsistency in performance. To overcome this, it was postulated that the birds must be guided in their selection by controlling the quantity of the diet offered. Also, the composition of the different diets fed sequentially or loose-mixed should not be too contrasting in energy, protein, and calcium.

The objective of this work was to evaluate the performance of laying hens habituated before point of lay to consume whole wheat and balancer diet in loose-mix or in sequential feeding systems. They were compared with conventional feeding using a single complete diet. Controlled quantities of these dietary components were fed in both the habituation and experimental periods.

MATERIALS AND METHODS

Habituation Period (wk 16 to 18)

Laying birds need a period of learning before becoming proficient in selecting feedstuffs (Forbes and Covasa, 1995). The birds were habituated to the feeding methods using wheat grains and a balancer diet from wk 16 to 18 of age. The objective was to particularly adapt the birds in sequential feeding to whole wheat intake before point of lay (Umar Faruk et al., 2008). The birds were housed individually in bottom-wired cages (25 × 38 cm), equipped with nipple drinkers and individual feeders. This was to allow for an individual follow-up of birds during this period.

The control treatment was fed a single control growing diet (Table 1) containing 2,800 kcal of ME/kg and 16% CP. The loose-mix and the sequential groups were fed whole wheat and a balancer growing diet containing 2,633 kcal of ME/kg and 19% CP. The total quantity of offered diet was progressively increased from 70 to 83 g/bird per day from wk 16 to 18, respectively. The quantity of the balancer growing diet, fed in loose-mix and in sequential groups, was 65% of the total diet given daily. Wheat represents 35% on the assumption that if the birds consumed all of the offered diet, they will therefore consume a similar amount of daily nutrients as the control. In sequential feeding, the wheat was fed in the morning followed by the balancer diet in the afternoon. The wheat was offered 4 h after lights-on for a period of 2 and 3 h during wk 16 and 17, respectively. In loose-mix, the wheat and the balancer diet were mixed and the mixture was fed in the same feeding trough.

Experimental Period (wk 19 to 46)

The 3 feeding systems studied and the hours of feed distribution are illustrated in Figure 1. The experimental period was from wk 19 to 46. During this period, the birds were housed in wire-bottomed cages (550 cm^2/hen) designed to accommodate 5 birds per cage. Each of the 3 treatments was allocated 80 birds divided into 16 cages as replicates. Body weight was used as the criterion for placement such that homogeneous mean BW was placed per cage and per treatment. The birds received 16 h of light/d throughout the experimental period and water was fed ad libitum. Daily temperature was maintained at 21.7 ± 0.7°C.

The control treatment was fed the control layer diet containing 2,750 kcal of ME/kg and 17.5% CP (Table 1). The sequential and loose-mix groups received the balancer layer diet containing 2,380 kcal of ME/kg, 23% CP, and 7.2% calcium (finely ground) sequentially or in loose-mix with whole wheat. This diet was formulated to reach the daily nutritional balance as the control diet, assuming a ratio of 50% wheat and 50% balancer diet. Thus, in sequential and loose-mix treatments, 60.5 g of the diet was fed as whole wheat and the remaining 60.5 g as balancer diet. Each bird received 121 g/d of diet corresponding to 105% of the breeder's guidelines (ISA, 2007).

All of the birds received their daily ration in 2 distributions at 4 and 11 h after lights-on respectively. In sequential feeding, wheat was fed at first distribution, whereas the balancer diet was fed at second distribu-

Table 1. Composition of experimental diets

Item	Habituation period (16 to 18 wk) Control growing	Habituation period (16 to 18 wk) Balancer diet growing	Experimental period (19 to 46 wk) Control laying	Experimental period (19 to 46 wk) Balancer diet laying	Whole wheat
Ingredient (%)					
Wheat	34.66	—	50.00	—	
Maize	35.00	53.57	16.13	32.08	
Wheat bran	10.00	15.31	2.54	5.01	
Maize gluten	—	—	3.29	6.62	
Soybean meal	16.50	25.25	16.97	34.08	
Soybean oil	—	—	0.80	1.60	
Calcium carbonate	1.84	2.82	8.00	16.04	
Bicalcium phosphate	1.09	1.67	1.16	2.33	
Refined salt	0.20	0.31	0.20	0.40	
Sodium bicarbonate	0.20	0.31	0.20	0.40	
L-Lysine 78	—	—	0.11	0.22	
DL-Methionine	0.01	0.02	0.11	0.22	
Premix[1]	0.50	0.77	0.50	1.00	
Calculated composition (%)					
ME (kcal/kg)	2,800	2,633	2,750	2,380	3,120
CP	16.05	18.33	17.52	23.00	11.90
DM	87.74	87.48	89.06	89.88	86.80
Fat	2.36	2.90	2.51	3.67	1.35
Ash	5.75	8.06	11.71	22.07	1.60
Crude fiber	3.59	4.10	3.01	3.37	2.65
Lysine	0.72	0.93	0.81	1.31	0.31
Methionine	0.32	0.39	0.45	0.71	0.20
Calcium	1.20	1.82	3.61	7.20	0.03
Total phosphorus	0.56	0.71	0.53	0.76	0.32

[1]Vitamin and mineral premix supplied the following amounts per kilogram of premix: vitamin A, 1,600,000 IU; vitamin D_3, 480,000 IU; vitamin E, 2,000 mg; vitamin K_3, 400 mg; vitamin B_1, 109 mg; Zn, 11,000 mg; Mn, 12,000 mg; Cu (sulfate), 1,200 mg; Fe, 4,000 mg; I, 200 mg; Se, 60 mg; DL-methionine, 120 g; and canthaxanthin, 200 mg.

tion after the removal of wheat from the trough using an electric vacuum cleaner (Dyson DC19 vacuum cleaner, Dyson Limited, Malmesbury, UK). In loose-mix and control, the same diets were fed during the 2 distributions. The first distribution was made 4 h after lights-on so as to avoid a negative correlation between feed intake and oviposition (Ballard and Biellier, 1969; Nys et al., 1976). The quantity of wheat offered was based on the conclusions of Bennet (2003) that whole grains should not exceed 50% of the total diet offered to avoid the condition whereby hens will have difficulty in finding the protein concentrate in the ration.

Total feed intake was measured weekly as the difference between feed offered and leftover. In sequential feeding, wheat intake was measured by directly measuring leftover wheat. In loose-mix, wheat intake was measured after separating the wheat from the balancer diet using a manual sieve (2 mm diameter).

Birds were weighed individually at wk 16, 19, 27, 37, and 46. Egg production was measured by recording the

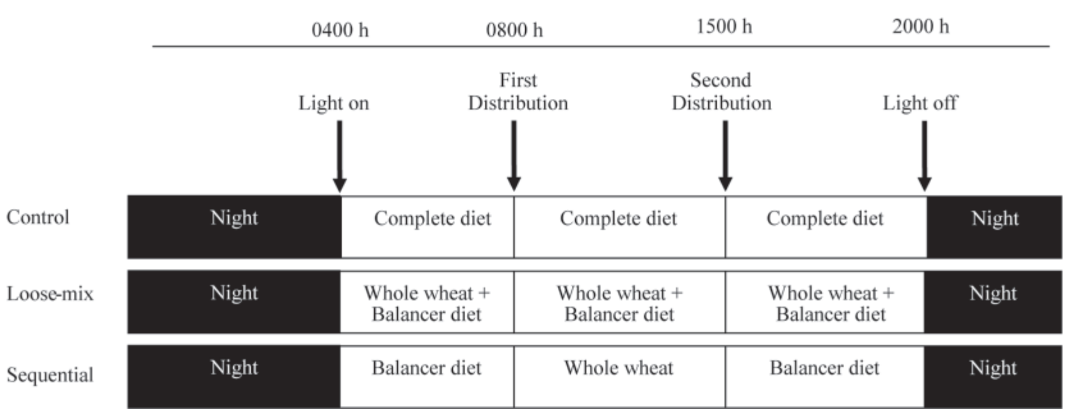

Figure 1. Illustration of the 3 feeding systems (control, loose-mix, and sequential) and the hour of feed distribution. During first distribution, half of the total daily ration was offered, and whole wheat was offered at this time for the sequentially fed birds. The remainder of the total daily ration was fed during the second distribution. The balancer diet was fed at this time for the sequentially fed birds. All feeders were emptied before each distribution.

number of eggs produced daily. Individual egg weight was recorded daily. The weights of the egg yolk, albumen, and shell were measured every 4 wk starting from wk 21 of age. To measure these components, all eggs produced on a given day of the week were collected, weighed individually, and then broken. The albumen and the chalazae were separated from the yolk using forceps before weighing the yolk. The shells were carefully washed and dried for 12 h in a drying oven at 70°C and then weighed. All measurements were taken to the nearest 0.01 g.

The weights of the principal digestive organs were taken at wk 19 and 46. For this measurement, 8 and 16 birds per treatment were used at wk 19 and 46, respectively. At wk 46, eight birds were killed in the morning (0800 h), whereas the remaining 8 birds were killed in the afternoon (1500 h). This was to allow for an evaluation of the effect of daily feeding rhythm on the hepatic lipid, protein, and glycogen contents. The birds were first weighed before being injected with sodium pentobarbital solution (1 mL/kg; CEVA Santé Animale–La Ballastière, Libourne, France). The abdominal cavity was then dissected and the digestive tract was collected and separated into its different segments: proventriculus, gizzard, duodenum, pancreas, jejunum, and ileum. These digestive segments were first emptied and dried using a paper towel before weighing. The proventriculus and the gizzard were placed in an iced container (−4°C) for 3 h to facilitate the removal of the surrounding fat before being emptied. Hepatic lipid (%/liver) was measured according to the Folch procedure (Folch et al., 1957). Hepatic protein (g/g of liver) content was determined by bicinchoninic assay kit (Uptima, Interchim, Montluçon, France) according to procedures of Smith et al. (1985). Analysis of liver glycogen (mmol/g of liver) was conducted according to procedure described by Dalrymple and Hamm (1973) and adapted by Monin and Sellier (1985).

Metabolizable energy requirement (kcal/bird per d) was estimated using the predictive equation of Sakomura (2004):

$$\mathrm{ME} = \mathrm{W}^{0.75} \times (165.74 - 2.37 \times \mathrm{T}) + 6.68 \times \mathrm{WG} + 2.40 \times \mathrm{EM},$$

where ME = ME requirement (kcal/bird per d); T = temperature (°C); WG = weight gain (g/bird per d); EM = egg mass (g/bird per d); and W = BW (kg).

Protein requirement (g/bird per d) was determined using the predictive protein requirements equation (Sakomura et al., 2002):

$$\mathrm{PB} = 1.94 \times \mathrm{W}^{0.75} + 0.480 \times \mathrm{WG} + 0.301 \times \mathrm{EM},$$

where PB = protein requirement (g/bird per d); W = BW (kg); WG = weight gain (g/bird per d); and EM = egg mass (g/bird per d).

Statistical Analysis

Average values from cages were analyzed using StatView (version 5, SAS Institute Inc., Cary, NC). Collected data were analyzed based on 3 periods related to egg production stage (1) before peak, from 19 to 26 wk of age; (2) at peak, from 27 to 37 wk of age; and (3) after peak, from 38 to 46 wk of age. These weeks (19, 26, 37, and 46) also coincide with the weeks in which BW was measured in this work.

A 1-way ANOVA according to the GLM model below was used to test treatment effect on feed intake, egg production and weight, egg components weight, BW, and digestive organs weight:

$$\mathrm{Y_{ij}} = \mathrm{R_i} + \varepsilon_{\mathrm{ij}},$$

where $\mathrm{Y_{ij}}$ = measured variables for regimen i and cage j; $\mathrm{R_i}$ = regimen effect (i = sequential, loose-mix, control) and j being the cage number in regimen i; and $\varepsilon_{\mathrm{ij}}$ = residual.

The hepatic lipid, protein, and glycogen contents were subjected to 2-way ANOVA using the following model:

$$\mathrm{Y_{ijk}} = \mathrm{R_i} + \mathrm{H_j} + \varepsilon_{\mathrm{ijk}},$$

where $\mathrm{Y_{ijk}}$ = measured variables for regimen i, hour of slaughter j, and cage k; $\mathrm{R_i}$ = regimen effect (i = sequential, loose-mix, control); $\mathrm{H_j}$ = slaughter time effect (j = morning, afternoon) and k being the cage number in regimen i and hour j; and $\varepsilon_{\mathrm{ijk}}$ = residual. Results were considered different if $P < 0.05$, and Bonferroni-Dunnet pairwise comparison was used to compare differences in means.

RESULTS

Habituation Period (wk 16 to 18)

The overall average total feed intake from wk 16 to 19 was similar among the 3 treatments: 67.1, 67.4, and 66.3 g/bird per day for control, loose-mix, and sequential, respectively. Wheat intake in sequential feeding increased with increasing age and the quantity offered. It was 12 and 38 g/bird per day for wk 16 and 18, respectively. Body weight gain was similar among the treatments: 8.1, 8.6, and 8.4 g/bird per day for control, loose-mix, and sequential, respectively.

Experimental Period (wk 19 to 46)

As indicated in the statistical analysis section, data collected during the experimental period were analyzed based on 3 periods (before peak: 19 to 26 wk; at peak: 27 to 37 wk; and after peak: 38 to 46 wk). At the onset of the experimental period, 1 replicate belonging to the control group was eliminated from the study due to technical reasons. This reduced the number of replicates in this treatment to 15, whereas sequential and loose-mix each had 16.

The overall average total feed intake during the experimental period (Table 2) was found to be lower ($P < 0.01$) in sequential feeding (108.7 g/bird per d) when compared with what was obtained with loose-mix (115.9 g/bird per d) and control (115.2 g/bird per d). Wheat intake was found to be lower ($P < 0.01$) in sequential feeding compared with loose-mix, with 51.2 and 60.2 g/bird per d representing about 47 and 50% of the total intake, respectively. Conversely, balancer diet intake was higher ($P < 0.01$) with sequential feeding (60.1 g/bird per d) compared with loose-mix (58.5 g/bird per d).

Similar egg production and egg weight were observed among the 3 treatments (Table 2). Similarly, the average egg mass was similar among treatments. Egg production and weight increased with increasing age; thus, the effect was consistent across the 3 periods. Feed conversion ratio (**FCR**) was lower ($P < 0.01$) for sequential (2.01) than loose-mix (2.21) and control (2.11); FCR of loose-mix and control FCR were not statistically different.

Body weight increased with age and was similar among treatments up to wk 26 (Table 2). However, a difference in BW was observed at wk 37. Sequentially fed birds were lighter in BW (1,724 g/bird) when compared with hens fed loose-mix (1,862 g/bird) and control (1,819 g/bird). Loose-mix was similar in BW to control. No increase in BW from wk 37 to the end of

Table 2. Feed consumption, egg production, egg weight, feed conversion ratio (FCR), and BW of sequential- and loose-mix-fed hens from 19 to 46 wk of age

	Treatment				
Measurement	Control	Loose-mix	Sequential	P-value	SEM
Average total feed intake (g/bird per d)					
19 to 26 wk	108.7[a]	109.0[a]	103.4[b]	<0.01	1.3
27 to 37 wk	117.4[a]	118.0[a]	109.6[b]	<0.01	0.8
38 to 46 wk	118.5[a]	119.4[a]	112.8[b]	<0.01	1.0
Overall	115.2[a]	115.9[a]	108.7[b]	<0.01	0.9
Average wheat intake (g/bird per d)					
19 to 26 wk	—	ND[1]	46.0	—	—
27 to 37 wk	—	60.0[a]	49.4[b]	<0.01	0.8
38 to 46 wk	—	60.4[a]	53.6[b]	<0.01	0.8
Overall[2]	—	60.2[a]	51.2[b]	<0.01	0.8
Average balancer diet intake (g/bird per d)					
19 to 26 wk	—	ND	57.4	—	—
27 to 37 wk	—	58.2[a]	60.2[b]	<0.01	0.3
38 to 46 wk	—	58.8[a]	60.0[b]	<0.01	0.4
Overall[2]	—	58.4[a]	60.1[b]	<0.01	0.3
Egg production (%)					
19 to 26 wk	86.9	82.9	87.1	NS[3]	1.2
27 to 37 wk	96.4	94.3	96.2	NS	0.9
38 to 46 wk	94.5	91.3	94.3	NS	1.5
Overall	93.1	90.2	93.1	NS	1.0
Egg weight (g)					
19 to 26 wk	53.6	53.5	53.2	NS	0.4
27 to 37 wk	60.4	61.1	60.3	NS	0.4
38 to 46 wk	62.4	61.9	62.1	NS	0.4
Overall	59.1	59.2	58.8	NS	0.4
Egg mass (g/d)					
19 to 26 wk	46.9	44.8	46.9	NS	0.8
27 to 37 wk	58.3	57.4	58.1	NS	0.7
38 to 46 wk	59.0	57.0	59.0	NS	1.1
Overall	55.2	53.6	55.0	NS	0.8
FCR (g of feed:g of egg)					
19 to 26 wk	2.37[a]	2.53[b]	2.30[a]	<0.01	0.04
27 to 37 wk	2.02[a]	2.07[a]	1.89[b]	<0.01	0.03
38 to 46 wk	2.02[ab]	2.13[b]	1.93[a]	<0.01	0.04
Overall	2.11[a]	2.21[a]	2.01[b]	<0.01	0.03
BW (g)					
19 wk	1,504	1,555	1,522	NS	22.5
26 wk	1,716	1,720	1,655	NS	27.7
37 wk	1,819[a]	1,862[a]	1,724[b]	<0.01	26.1
46 wk	1,823[a]	1,862[a]	1,723[b]	<0.01	26.1

[a,b]Values within the same line with no common superscripts differ significantly ($P < 0.05$).

[1]The respective intakes of wheat and balancer diet between wk 19 to 26 were not available for loose-mix treatment.

[2]Overall intakes of wheat and balancer diet were for values from wk 27 to 46.

[3]Not significant ($P > 0.05$).

the experimental period was observed and hen weight in the sequential feeding group remained lower than the 2 other groups ($P < 0.01$).

In sequential feeding, overall yolk weight (both in g and %) was similar to that of control but inferior to loose-mix (Table 3). Control was similar to loose-mix in yolk weight. Similar egg yolk weight was obtained among treatments before and after peak in egg production (wk 19 to 26 and 38 to 46, respectively). Yolk weight during the peak period (wk 27 to 37) was inferior in sequential compared with loose-mix, but the 2 treatments were similar to the control.

Overall, sequential feeding resulted in heavier eggshell weight (g and %) compared with that obtained in hens fed loose-mix and control (Table 3). Eggshell weight was similar among treatments before peak. At peak, eggshell weight (g) was similar between sequential and loose-mix, and both were higher than the control. However, during this period, eggshell weight (%) was higher in sequential feeding followed by loose-mix and control in descending order. Eggshell weight (%) was similar between control and sequential treatment after peak. There was no treatment effect on the albumen weight (both in g and %) throughout the experimental period (Table 3).

At the end of the habituation period (wk 19), significant differences in the relative weight of digestive organs were observed only for the duodenum and ileum (Table 4). Sequential feeding resulted in heavier duodenum and ileum when compared with loose-mix and control. At the end of the experimental period (wk 46), sequential feeding resulted in heavier gizzard, liver, and pancreas. However, proventriculus, duodenum, ileum, and jejunum weights were similar among treatments at this period. However, no effect of slaughter hour was observed on the weight of these organs. Likewise, no interaction between slaughter hour and treatment on the weight of digestive organs was observed.

Hepatic glycogen content (mmol/g of liver) was similar between sequential and control (Figure 2). However, it was higher in loose-mix compared with the 2 other treatments. In all of the treatments, birds killed in the morning had lower glycogen content compared with those killed in the afternoon. Similar hepatic protein (g/g of liver) content was observed in all of the treatments (Figure 3). Hepatic protein content was affected by slaughter hour; hence, it was higher for birds killed in the morning. Liver lipid content was not affected by treatment or slaughter hour (Figure 4).

DISCUSSION

During the habituation period, increased wheat intake with increasing age and quantity offered indicated a successful adaptation of the birds to consuming whole cereals. This confirms our hypothesis obtained from a previous study (Umar Faruk et al., 2008), in which we observed low wheat intake at wk 25, due to sudden in-

Table 3. Weight (g) and proportion (%) of egg yolk and shell of laying hens fed whole wheat sequentially or in loose-mix from 19 to 46 wk of age

Measurement	Unit	Treatment				
		Control	Loose-mix	Sequential	P-value	SEM
Egg yolk						
19 to 26 wk	g	11.4	11.5	11.2	NS[1]	0.13
	%	21.4	21.7	21.2	NS	0.20
27 to 37 wk	g	14.8^{ab}	15.1^{a}	14.3^{b}	<0.01	0.15
	%	24.9^{ab}	25.1^{a}	24.1^{b}	<0.01	0.24
38 to 46 wk	g	16.2	16.2	16.0	NS	0.14
	%	25.9	26.3	25.6	NS	0.22
Overall	g	14.4^{ab}	14.5^{a}	14.1^{b}	<0.01	0.10
	%	24.3^{ab}	24.6^{a}	23.9^{b}	<0.01	0.18
Eggshell						
19 to 26 wk	g	5.5	5.5	5.6	NS	0.06
	%	10.4	10.4	10.6	NS	0.09
27 to 37 wk	g	5.5^{a}	5.9^{b}	6.1^{b}	<0.01	0.08
	%	9.3^{a}	9.8^{b}	10.3^{c}	<0.01	0.11
38 to 46 wk	g	6.2^{ab}	6.1^{a}	6.4^{b}	<0.01	0.06
	%	10.0^{ab}	9.9^{a}	10.3^{b}	<0.01	0.09
Overall	g	5.8^{a}	5.9^{a}	6.1^{b}	<0.01	0.05
	%	9.9^{a}	10.0^{a}	10.4^{b}	<0.01	0.07
Egg albumen						
19 to 26 wk	g	36.2	35.7	35.6	NS	0.37
	%	68.3	67.9	67.6	NS	0.30
27 to 37 wk	g	39.2	39.4	38.7	NS	0.41
	%	65.8	65.1	65.1	NS	0.31
38 to 46 wk	g	39.8	39.1	39.8	NS	0.38
	%	64.0	63.7	64.1	NS	0.25
Overall	g	38.6	38.2	38.3	NS	0.36
	%	65.7	65.3	65.4	NS	0.26

[a–c]Values within the same line with no common superscripts differ significantly ($P < 0.05$).

[1]Not significant ($P > 0.05$).

Table 4. Effect of sequential and loose-mix feeding on the weight of digestive organs (% BW) at wk 19 and 46 of hens fed whole wheat sequentially or in loose-mix with a balancer diet from 16 to 46 wk of age

Organ (% BW)	Week 19[1]					Week 46[2]				
	Control	Loose-mix	Sequential	*P*-value	SEM	Control	Loose-mix	Sequential	*P*-value	SEM
Proventriculus	0.31	0.27	0.31	NS[3]	0.01	0.35	0.31	0.35	NS	0.01
Gizzard	1.84	2.16	2.19	<0.05	0.10	1.21[b]	1.38[ab]	1.46[a]	<0.05	0.05
Duodenum	0.54[ab]	0.51[b]	0.64[a]	<0.05	0.03	0.57	0.56	0.58	NS	0.02
Jejunum	0.85	0.85	1.00	<0.05	0.05	0.94	0.90	0.95	NS	0.03
Ileum	0.63[b]	0.62[b]	0.75[a]	<0.05	0.03	0.76	0.72	0.70	NS	0.03
Liver	2.47	2.43	2.51	NS	0.13	2.78[b]	2.96[ab]	3.10[a]	<0.05	0.08
Pancreas	0.21	0.22	0.22	NS	0.01	0.16[b]	0.18[ab]	0.19[a]	<0.05	0.01

[a,b]Values within the same line with no common superscripts differ significantly ($P < 0.05$).

[1]Eight birds/treatment were killed at wk 19. All of the birds were killed at the same hour (0800 h).

[2]Sixteen birds/treatment were killed at wk 46. Eight were killed in the morning (0800 h) and the remaining 8 birds in the afternoon (1500 h). No slaughter hour effect ($P > 0.05$) was observed on the measured measurements.

[3]Not significant ($P > 0.05$).

troduction of wheat grains in the diet of birds fed mash up to this age. Therefore, a learning period remains necessary when birds are to be fed with wheat grains (Forbes and Covasa, 1995).

Total feed intake was reduced when diets were fed sequentially. Blair et al. (1973) observed an increased feed intake in the sequential treatment compared with the control. However, they fed pellet balancer diet ad libitum as compared with mash balancer diet fed in controlled quantity in the present work. Reduced feed intake of sequentially fed birds in this work agreed with reports of Leeson and Summers (1978), even though the morning diet was both high in protein and energy whereas the afternoon diet was low in these nutrients. Our results also agreed with Reichmann and Connor (1979), and Lee and Ohh (2002), who had an experimental design similar to our study.

In the present study, low feed intake in sequential feeding was a result of reduced wheat intake. Wheat intake was significantly lowered (−9 g/bird per d) in the sequential than in the loose-mix treatment. Higher wheat intake in loose-mix may be attributed to the feed particle selection (Picard et al., 1997; Umar Faruk et al., 2008). Increasing particle size increases feed intake (Safaa et al., 2009). Particle selection is more likely to be seen in loose-mix because heterogeneity in particle

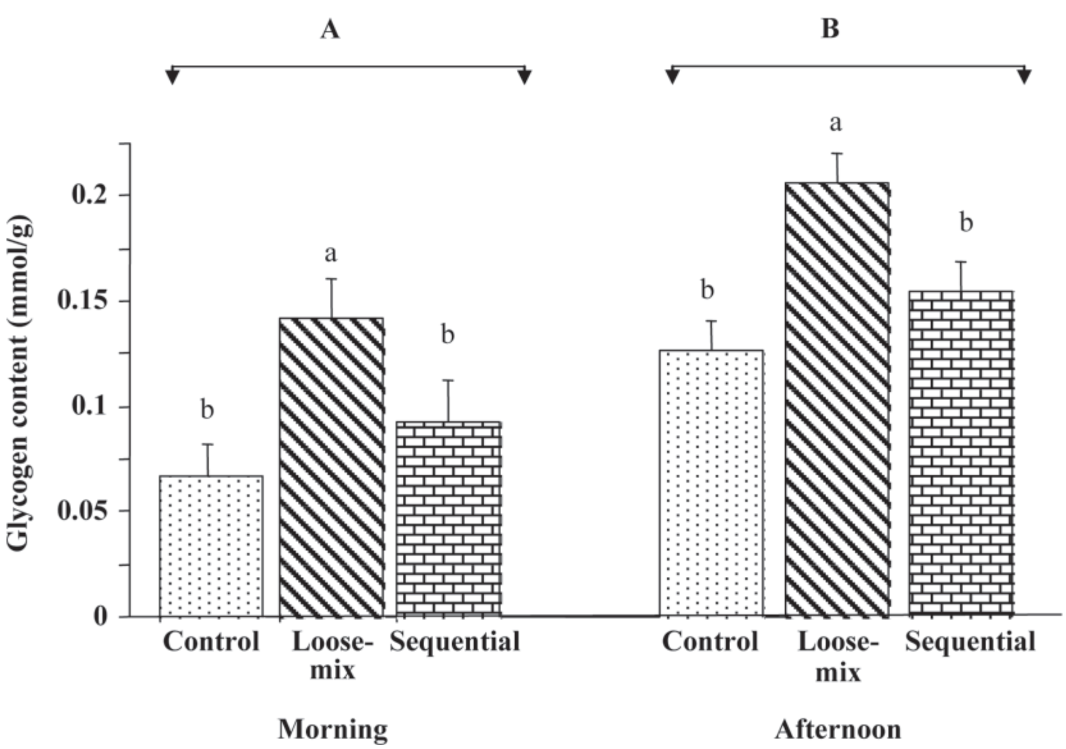

Figure 2. Glycogen content (mmol/g of liver) at wk 46 of birds fed a complete diet (control), whole wheat and balancer diet together (loose-mix), or whole wheat and balancer diet alternately (sequential). Eight birds each were killed in the morning and afternoon. Lowercase letters indicate differences between treatments, whereas uppercase letters indicate differences between the time of day (morning vs. evening).

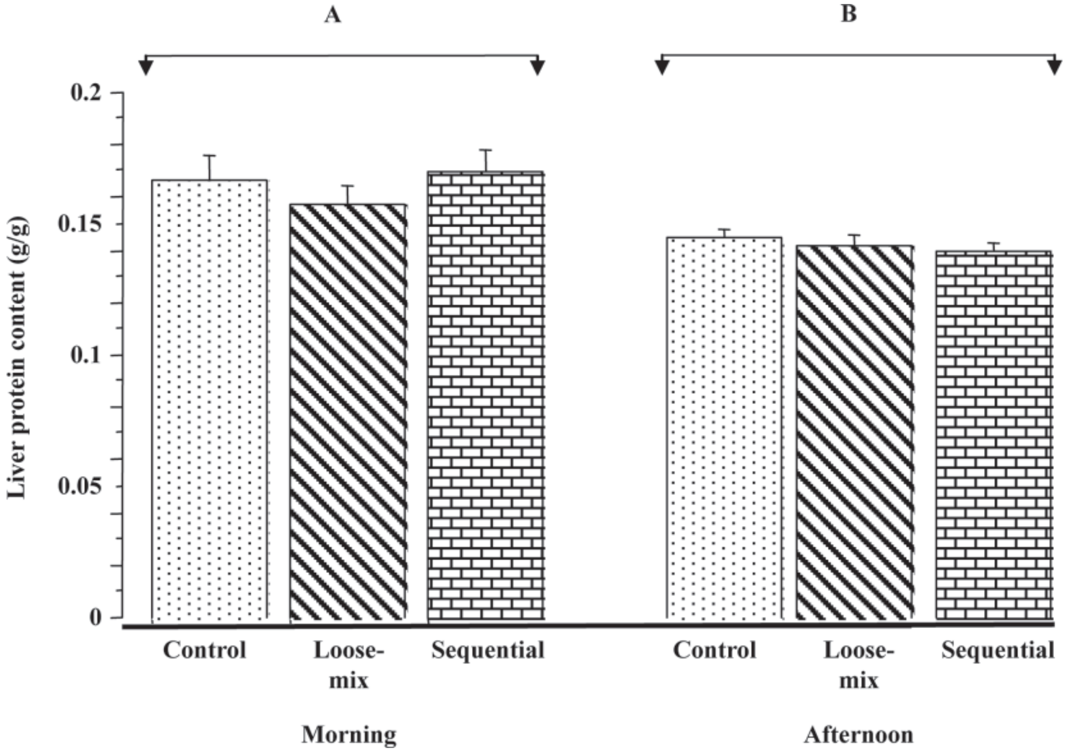

Figure 3. Liver protein content (g/g of liver) at wk 46 of laying hens fed a complete diet (control), whole wheat and balancer diet together (loose-mix), or whole wheat and balancer diet alternately (sequential). Eight birds were killed both in the morning and afternoon. Uppercase letters indicate the difference between morning and afternoon. The absence of lowercase letters indicates no difference between treatments.

size is increased by the addition of wheat grains. This, therefore, confirmed the suggestion of Bennet (2003) that only half of an offered diet should be in the form of grains, to avoid overconsumption of grains.

Although balancer diet intake was significantly higher (+1.7 g/bird per d) in sequential feeding, this was not enough to make up the difference in total feed intake. This was due to the limitation in the daily quantity of the diet offered (105% of the daily spontaneous feed consumption of the genotype currently used). Higher balancer diet intake in sequential feeding could be attributed to its protein and mineral (especially calcium) contents. These were amplified by the time of the day at which this diet was offered. The pattern of daily feed

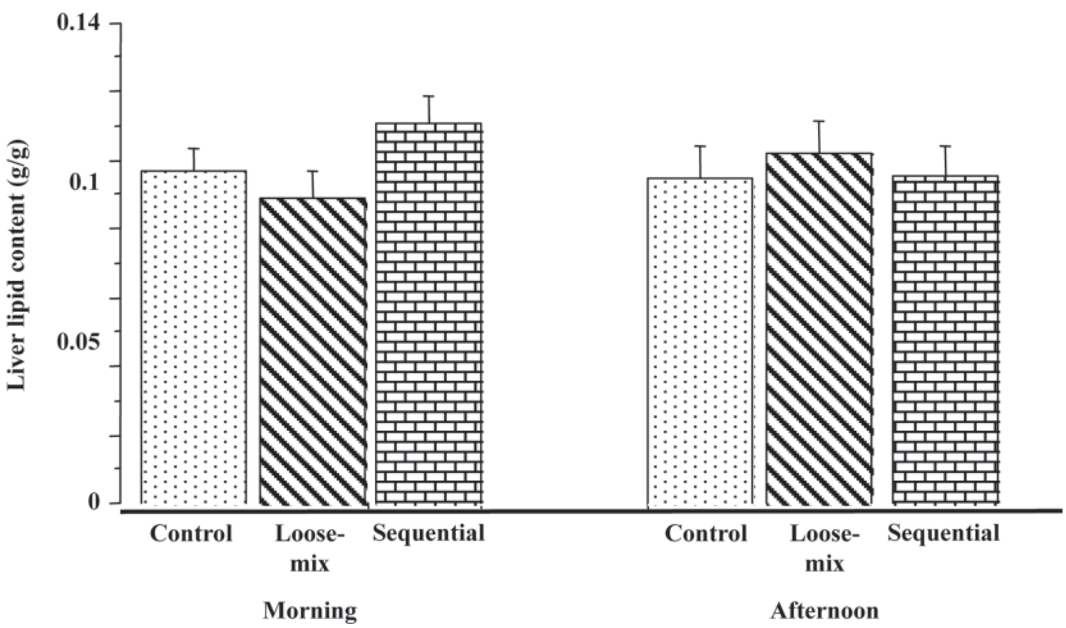

Figure 4. Liver lipid content (g/g of liver) at wk 46 of laying hens fed a complete diet (control), whole wheat and balancer diet together (loose-mix), or whole wheat and balancer diet alternately (sequential). Eight birds were killed both in the morning and afternoon.

Table 5. Calculated ME (kcal/bird per d) and protein (g/bird per d) intakes and requirements of laying hens fed whole wheat sequentially or in loose-mix from 19 to 46 wk of age

Measurement	Daily intake level	Treatment			*P*-value	SEM
		Control	Loose-mix	Sequential		
ME (kcal/bird per d)						
19 to 26 wk	Intake[1]	299.0^{a}	$299.8^{a,2}$	280.1^{b}	<0.01	3.7
	Requirement[3]	304.4	295.1	291.9	NS[4]	3.6
	Difference[5]	-5.4^{a}	4.7^{b}	-11.8^{a}	<0.01	2.0
27 to 37 wk	Intake	322.9^{a}	325.7^{a}	297.6^{b}	<0.01	2.4
	Requirement	322.8^{ab}	325.8^{a}	313.6^{b}	<0.01	2.7
	Difference	0.1^{a}	-0.1^{a}	-16.0^{b}	<0.01	1.8
38 to 46 wk	Intake	325.9^{a}	328.1^{a}	309.8^{b}	<0.01	2.5
	Requirement	322.6	319.6	313.9	NS	3.3
	Difference	3.3^{ab}	8.5^{a}	-4.1^{b}	<0.01	2.5
Overall (19 to 46 wk)	Intake	317.0^{a}	319.1^{a}	296.5^{b}	<0.01	2.6
	Requirement	317.5	315.0	307.5	NS	2.9
	Difference	-0.5^{a}	4.0^{a}	-11.0^{b}	<0.01	1.8
Protein (g/bird per d)	Intake	19.0	19.0	18.7	NS	0.1
19 to 26 wk	Requirement	18.9^{a}	17.9^{b}	18.1^{ab}	<0.01	0.3
	Difference	0.1^{a}	1.1^{b}	0.6^{ab}	<0.05	0.2
27 to 37 wk	Intake	20.6^{a}	20.5^{a}	19.7^{b}	<0.01	0.1
	Requirement	21.2	21.2	21	NS	0.2
	Difference	−0.6	−0.6	−1.0	NS	0.2
38 to 46 wk	Intake	20.8^{a}	20.7^{a}	20.2^{b}	<0.05	0.1
	Requirement	20.8	20.1	20.5	NS	0.3
	Difference	−0.1	0.6	−0.4	NS	0.3
Overall (19 to 46 wk)	Intake	20.2^{a}	20.2^{a}	19.6^{b}	<0.05	0.1
	Requirement	20.4	19.9	20.0	NS	0.2
	Difference	−0.2	0.3	−0.4	NS	0.2

[a,b]Values within the same line with no common superscripts differ significantly ($P < 0.05$).

[1]Metabolizable energy and protein intakes were calculated by multiplying the quantity of diet consumed and the calculated ME and protein contents of the diet, respectively (Table 1).

[2]Due to the nonavailability of actual intake of wheat and balancer diet during the period from wk 19 to 26, energy and protein intakes were estimated based on equal intake of wheat and balancer diet during this period. This concerns only birds fed in loose-mix.

[3]Requirements in ME and protein were calculated according to Sakomura (2004) and Sakomura et al. (2002), respectively. The temperature values were used according to actual temperatures for each period (i.e., 21.82, 22.00, and 21.28°C for periods 19 to 26, 27 to 37, and 38 to 46 wk, respectively).

[4]Not significant ($P > 0.05$).

[5]Difference between requirements and intakes was calculated as the intake minus the requirement.

intake in laying hens is influenced by the egg-forming cycle as well as by photoperiod (Ballard and Biellier, 1969; Nys et al., 1976; Choi et al., 2004). Thus, hens consumed more diet in the afternoon (Keshavarz, 1998), mainly to account for calcium required in eggshell formation, especially on egg-forming days (Mongin and Sauveur, 1974).

In our work, egg production and weight increased with hen age and were consistent with the breeders' guidelines (ISA, 2007). We observed similar egg production and egg weight among all 3 treatments. This indicates that the reduction in feed intake of sequentially fed birds had no effect on their egg production, egg weight, and mass during the period of study. Blair et al. (1973), Reichmann and Connor (1979), and Lee and Ohh (2002) all reported similar egg production when hens were fed diets sequentially. Robinson (1985) reported a decrease in egg production related to the difficulty in timing protein meal at a particular time of the day. Conversely, Leeson and Summers (1978) reported an increase in egg production due to increase in protein and energy intake of birds fed sequentially. Egg weight was similar (Blair et al., 1973) or reduced (Leeson and Summers, 1978; Robinson, 1985; Lee and Ohh, 2002) in sequential compared with conventional feeding. The latter authors reported decreased egg weight of birds fed in loose-mix. Egg weight and rate of lay are reduced when protein intake is reduced (Morris and Gous, 1988). In the present work, overall protein intake was estimated to be statistically higher for hens fed control (20.2 g/bird per d) and loose-mix (20.2 g/bird per d) compared to the sequential (19.6 g/bird per d) treatment (Table 5). This difference in protein intake had no effect on egg production and weight because the estimated daily intake of protein was similar to the estimated daily requirements in all 3 treatments.

The reduced intake, while maintaining similar egg production and weight in sequential feeding, resulted in a consistent improvement of FCR compared with the 2 other treatments: 5 and 10% relative to control and loose-mix, respectively.

There was no treatment effect on growth performance during the prelaying stage. Cowan et al. (1978) and Karunajeewa and Tham (1984) reported no difference in BW between hens fed a choice of whole grains and a protein concentrate and those fed a control complete diet during the prelaying period. As a result of a lower BW gain from wk 19 to 37, sequentially fed birds had low BW at the end of the experimental period (wk 46). Body weight gain during this period was significantly lower in sequential feeding (1.6 g/d) than loose-mix (2.4 g/d) and control (2.4 g/d). The difference in BW tended to appear about a week after peak production (wk 26), suggesting that body deposition was lowered in sequential feeding to balance the feed intake and the demand in energy required for egg production (Scanes et al., 1987). Estimation of energy intake (Table 5) indicated that sequentially fed birds ingested less energy compared with loose-mix and control. Similar energy intake was observed between loose-mix and control. Energy intake compared with their respective requirements showed a consistent balance for loose-mix and control. However, for sequential feeding, ME intake was lower than requirement. This suggested a slight increase in digestion efficiency in these birds because egg production performance was not affected. In sequential feeding, the reduction in ME intake agreed with results of Lee and Ohh (2002).

The proportion of egg components observed in this work was in line with the changes in the egg components reported by Harms and Hussein (1993). They observed that with increasing hen age, egg weight increases but the eggs contain proportionally more yolk and less albumen and shell. This is because the albumen weight with hen age increases but at a decreasing rate, whereas the yolk increases at a faster rate (Johnston and Gous, 2007). Percentage egg yolk was low in sequential feeding from 27 to 37 wk of age compared with the 2 other treatments. Increase in BW was also low in sequential feeding during this period. The difference in yolk weight was no longer significant at the end of the trial period as was also observed with BW gain. Dietary protein affected egg weight (Fisher, 1969) due to reduction in all components, but yolk and shell weight changed proportionately less than the total and albumen weight.

The percentage eggshell was found to be higher in sequential feeding. This was not surprising because calcium intake fed as flour was largely reinforced during the later part of the photoperiod, as a consequence of a higher level of calcium in the balancer diet compared with the control diet. Calcium absorption in laying hens is affected by stage of shell formation and is higher during the second part of the photoperiod (Etches, 1986; Nys, 1993; Kebreab et al., 2009). Because calcium supplied in this work was ground and mixed in the balancer diet, the results also confirmed the findings of Balnave and Abdoellah (1990) that granular sources of calcium are not an essential prerequisite in nutrient-fractioned feeding systems such as sequential feeding.

At the end of the experimental period, sequentially fed birds had heavier gizzards, pancreas, and liver. Dietary particle size is known to influence the avian digestive tract such that the gizzard weight increases with increasing particle size (Nir et al., 1990). Increase in gizzard weight of sequential and, to some extent, loose-mix-fed birds suggests an increase in grinding capacity compared with control. It could be suggested that this increased grinding capacity in sequential feeding allowed the efficient utilization of feed and this could explain to some extent the improved performance. Change in liver weight agreed with observations of De Basilio et al. (2001), who, under warm conditions, observed heavier livers in broiler birds fed sequentially compared with control. Karunajeewa (1978) observed similar liver weight between birds fed a choice of whole wheat and those fed control.

Similar liver DM and lipid contents between diets were in agreement with Karunajeewa (1978). According to Maurice and Jensen (1979), liver lipid content is affected by type and quantity of dietary cereal. It was not expected to currently differ among treatments because we only introduced 1 type of cereal. Wolford and Polin (1974) observed that increase in feed intake of birds resulted in increased liver lipid content. We observed no difference in hens fed either loose-mix or sequential feeding, although the former had higher feed intake than the latter. Glycogen content is generally believed to vary in function of the feeding state of the animal (Greenberg et al., 2006). Birds killed in the afternoon had higher hepatic glycogen and this was related to their feeding status and also the presence of the digestive enzyme amylase (Rideau et al., 1983). This author showed that amylase concentration is high between 4 and 10 h after oviposition. In this work, the birds were killed in the period corresponding to the peak presence of amylase. Modification in liver glycogen content between groups suggested that changes in liver glucose utilization occurred. This may result from a better digestive utilization (grinding capacity, starch digestion, as well as involvement of gastrointestinal hormones) as suggested by the higher weight of gizzard and pancreas in both sequential and loose-mix groups. The significant effect with the loose-mix group may result from the higher intake of wheat as compared with the sequential group.

However, feeding management (Van Krimpen et al., 2005), especially sequential feeding (Jordan et al., 2009), may affect feather pecking in laying hens, possibly due to reduction in time spent on feed intake. There is, therefore, the need to investigate the optimal duration of the sequence (wheat-balancer diet) in sequential feeding. Equally, the quantity, form (ground or unground), and type of cereal [millet, sorghum, and maize (*Zea mays*)] to be offered need to be explored. It is also necessary to better understand the changes in digestive and liver functions based on feeding system. Sequential feeding imposes constraints in terms of feed allowance, but in particular, conditions can largely improve feed conversion. This feeding system is therefore a promising one in terms of performance but also in facilitating use of local feedstuffs introduced as whole grains.

This trial covers the first half of the egg production cycle; thus, it could be concluded for this period that when birds were fed a controlled quantity of whole wheat and balancer diet sequentially or loosely mixed, similar egg production performance was observed. Loose-mix resulted in similar performance to the classic feeding in terms of feed intake and efficiency of feed utilization, whereas sequential feeding largely increased feed efficiency. This can be used to advantage in reducing feeding cost.

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